

Full Length Research Paper

Evaluation of the stem bark of *Pistacia integerrima* Steud ex Brandis for its antimicrobial and phytotoxic activities

Shafiq ur Rahman^{1,2*}, Muhammad Ismail², Naveed Muhammad², Farhat Ali¹, kamran Ahmad Chishti¹ and Muhammad Imran³

¹Department of Pharmacy, Sarhad University of Science and Information Technology, Pakistan.

²Department of Pharmacy, University of Peshawar-25120, Pakistan.

³School of Pharmacy University of Lahore, 24 avenue blue area Islamabad, Pakistan.

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The antimicrobial and phytotoxic activities of the crude methanolic extract and its subsequent solvent fractions of *Pistacia integerrima* bark were investigated. The outstanding activity was shown by the ethyl acetate fraction followed by aqueous fraction against *Staphylococcus aureus* having zone of inhibition 19 and 15 mm respectively. The ethyl acetate fraction was also effective against *hoteus vulgaris* having zone of inhibition 15 mm. The outstanding minimum inhibitory concentration (MIC) was observed against *Staphylococcus aureus* by ethyl acetate (0.31 mg/ml) and *Salmonella typhi* by hexane fraction (0.37 mg/ml). The crude methanolic extract and subsequent solvent fractions were also tested against two fungal strains, that is, *Candida albicans* and *Aspergillus niger* no outstanding antifungal activity was found. All the fractions are good herbicidal and weedicidal at high concentration, however the considerable activity was shown by ethyl acetate (90% growth inhibition) followed by chloroform (70% growth inhibition) and methanol (60% growth inhibition) at a concentration of 500 ppm. The ability of crude methanol extract and its subsequent solvent fractions, specially the ethyl acetate fraction, to inhibit the growth of microorganism and plant *Lemna minor* L as an indication of its antimicrobial and phytotoxic potential. This provides a baseline for isolation and identification of new antimicrobial and phytotoxic compounds to the world of medicine.

Key words: *Pistacia integerrima* bark, antimicrobial, phytotoxic, minimum inhibitory concentration.

INTRODUCTION

Traditional medicines provide treatment for about 80% of the world population, especially in developing countries, indicating that about 3.5 to 4 billion people in the world rely on plants as a source of drug (Farnsworth et al., 1985; Patwardhan et al., 2004). Infectious diseases are the leading causes of death throughout the world, accounting for nearly one half of all death in the tropical countries, which is also becoming a serious problem in developed countries (Shah et al., 2011). So far a lot of indigenous plants have been tested against various

microorganisms (Jones et al., 2000; Omer et al., 2000; Islam et al., 2001; Ficker et al., 2003) and showed considerable results.

A large number of secondary metabolites have been isolated from the medicinal plants having antimicrobial activities, which is the increasing demand of the present era due to the developing resistance of microorganism against synthetic drugs (White et al., 1998). Phytotoxicity assays has been an important approach for identifying plants that are likely to be a source of herbicidal compounds of interest (Ma et al., 2011). Several studies reported the phytotoxicity of different solvent extracts of plants such as methanol, ethanol chloroform etc (Turker Camper, 2002; Goncalves et al., 2009). *Pistacia integerrima* Stewart ex Brandis belongs to family Anacardiaceae, commonly known as kakar singhi (Hindi) Shnaie (Pushto).

*Corresponding author. E-mail: Shafiq_pharma01@yahoo.com.

The galls, leaves and bark of this plant are used in the traditional medicine for the treatment of fever, cough, asthma, diarrhea, jaundice, and snake bites (Padulosi et al., 2002; Jan et al., 2009). The phytochemical constituents of leaves of *P. integerrima* Stewart ex Brandis has been reported (Ahmad et al., 2008). Tetracyclic triterpenoids from galls of *P. integerrima* Stewart ex Brandis have been isolated. Analgesic and anti-inflammatory activities of gall have been reported (Ansari and Ali, 1996). The free radical scavenger activity of leaves of *P. integerrima* Stewart ex Brandis in hyperurecemia and gout has also been reported (Ahmad et al., 2008). Literature survey revealed that there is no scientific reported work on the antimicrobial and phytotoxic activities of the bark of the plant, therefore this study was carried out to evaluate the antimicrobial and phytotoxic effects of crude methanol and its subsequent solvent fractions of plant bark against some selected microorganism and plant *Lemna minor* L respectively, so as to establish a scientific ground for the use of the *Pistacia integerrima* bark and exploration of the plant bark for new active compounds.

MATERIAL AND METHODS

Plant material

Fresh stem bark of *P. integerrima* Stew ex Brandis was collected from district buner of Khyber pukhthunkhwa, Pakistan. The plant was collected in the month of April 2010 and was identified by Chairman, Department of Botany, Prof. Dr. Muhammad Ibrar University of Peshawaing oucher sample (No.10420Bot) was kept in the herbarium at Botany Department, University of Peshawar for reference.

Extraction and preparation of plant extracts

The stem bark was air dried at room temperature and pulverized by using electric grinder. The powdered plant material (5.4 kg) was soaked in commercial grade methanol for 14 days at room temperature and was subjected to occasional shaking. After 14 days methanol soluble materials were filtered. The filtrate were then concentrated at 40°C using rotary evaporator and kept in air tight container until required for use. This procedure was repeated three times. Crude methanolic extract weighing 250 g was suspended in 1000 ml of distilled water and filtered. The filtrate was then partitioned successively with hexane (3×600 ml), chloroform (3×600 ml), ethyl acetate (3×600 ml) and butanol (3×600 ml). The resultant solvent extracts were concentrated using rotary evaporator and kept in air tight container until use (Bashir et al., 2009). The crude methanolic extract and its subsequent solvent fractions were screened for antimicrobial and phytotoxic activities.

Antibacterial assay

The bacterial isolates were first grown in a nutrient broth for 18 h before use. Surface viable counting technique was used for average number of viable organism per ml of stock suspension. Each time fresh stock suspension was prepared using about 10^6 cfuml⁻¹ (Igbinosa et al., 2009). Standard bacterial suspension 0.6 ml was spread on sterile nutrient agar in Petri dishes. Wells are then

bored in to the agar using sterile cork borer 8 mm in diameter through micro titer pipette. Filled the well with 0.1 ml (10 mgml⁻¹) of each extract and allow standing for 2 h at room temperature and then incubated at 37°C. Control was set up in parallel using the solvents that were used to reconstitute the extracts. The zone of inhibition was observed after 24 h. The results were compared with standard drug streptomycin at a concentration of 0.5 mg/ml (Igbinosa et al., 2009; Shah et al., 2011).

Antifungal assay

The fungal isolates were allowed to grow on a saboraud dextrose agar at 25°C until they sporulated. The fungal spores were harvested, washed with sterile normal saline and were standardized to 10^8 cfuml⁻¹ (Shah et al., 2011). The antifungal activity was carried out in accordance with Igbinosa et al. (2009) and Shah et al. (2011).

Phytotoxicity assay

The crude methanol extract and its subsequent solvent fractions were screened for phytotoxicity against *Lemna minor* L. The medium was prepared by mixing various inorganic components in 900 ml of distilled water (for 1000 ml of medium) and KOH solution was added for the adjustment of pH at 6.0 - 7.0. The medium was autoclaved at 121°C for 15 min. Stock solution was prepared by dissolving 15 mg of test sample 1.5 ml of in ethanol. Nine flasks (three for each dilution) were inoculated with 1000, 100 and 10 µl of the stock solution for 500, 50 and 5 ppm. The solvent was then evaporated overnight under sterilized conditions. Each flask was supplemented with 20 ml of the medium. Thereafter, 10 plants each containing a rosette of three fronds, were added to each flask. One other flask, supplemented with solvent as control and reference plant growth inhibitor (Paraquat), served as a standard phytotoxic drug. The flasks were plugged with cotton and placed in growth cabinet for 7 days. On the 7th day, the number of fronds per flask was counted. Results were analyzed as growth inhibition in percentage (Saeed et al., 2010; Ghazala and Shameel, 2005).

RESULTS AND DISCUSSION

Antibacterial assay

The antibacterial action, that is, the zone of inhibition and minimum inhibitory concentration (MIC) is shown in Table 1 and Table 2, respectively. The methanolic extract and its subsequent solvent fractions were tested against three gram positive bacteria, that is, *Staphylococcus aureus*, *Proteus vulgaris* and *Bacillus subtilis* and three gram negative bacteria, that is, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi*. The antibacterial activity of all the samples were compared with the broad spectrum antibacterial drug, that is, Streptomycin. The outstanding activity was shown by the ethyl acetate followed by aqueous fraction against *S. aureus* having zone of inhibition 19 and 15 mm, respectively. The ethyl acetate was also effective against *P. vulgaris* having zone of inhibition 15 mm. It is clear from Table 1 that the plant extracts and especially the ethyl acetate fraction is very effective against gram positive bacteria as compare to gram negative bacteria. Methanolic extract is effective

Table 1. Antibacterial activity of *Pistacia integerrima* bark (Zone of inhibition in mm).

Solvent fraction (10 mg/ml)	Zone of inhibition(mm)					
	<i>Staphylococcus aureus</i>	<i>Proteus vulgaris</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>
Methanol	10 ± 0.22	12 ± 0.30	10 ± 0.19	14 ± 0.21	11 ± 0.18	12 ± 0.56
Aqueous	15 ± 0.23	12 ± 0.32	11 ± 0.20	12 ± 0.25	10 ± 0.20	11 ± 0.12
Ethyl acetate	19 ± 0.13	15 ± 0.28	12 ± 0.21	13 ± 0.27	10 ± 0.22	11 ± 0.11
Hexane	8 ± 0.11	13 ± 0.22	10 ± 0.23	9 ± 0.11	10 ± 0.23	5 ± 0.24
Streptomycin (0.5 mg/ml)	17 ± 0.11	16 ± 0.09	16 ± 0.00	19 ± 0.07	9 ± 0.02	15 ± 0.06

Table 2. Minimum inhibitory concentration of different solvent fractions of *Pistacia integerrima* bark (mg/ml).

Test organism	Minimum inhibitory concentration (MIC)				
	Methanol	Aqueous	Ethyl acetate	Hexane	Streptomycin (Std)
<i>Staphylococcus aureus</i>	5.00 ± 0.12	0.63 ± 0.21	0.31 ± 0.12	10.0 ± 0.23	0.08 ± 0.03
<i>Proteus vulgaris</i>	10.0 ± 0.20	5.00 ± 0.26	1.25 ± 0.50	5.00 ± 0.19	0.03 ± 0.01
<i>Bacillus subtilis</i>	5.00 ± 0.24	9.00 ± 0.25	8.00 ± 0.32	10.0 ± 0.12	0.07 ± 0.02
<i>Pseudomonas aeruginosa</i>	2.50 ± 0.21	10.0 ± 0.12	0.41 ± 0.33	0.61 ± 0.10	0.05 ± 0.01
<i>Escherichia coli</i>	6.00 ± 0.32	6.00 ± 0.23	1.20 ± 0.32	8.00 ± 0.13	0.79 ± 0.02
<i>Salmonella typhi</i>	5.00 ± 0.11	9.00 ± 0.11	0.625 ± 0.31	0.37 ± 0.14	0.07 ± 0.05

Table 3. Antifungal activity of *Pistacia integerrima* bark (Zone of inhibition in mm).

Test organism	Zone of inhibition(mm)				
	Methanol (10 mg/ml)	Aqueous (10 mg/ml)	Ethyl acetate (10 mg/ml)	Hexane (10 mg/ml)	Amphotericin B (0.5 mg/ml)
<i>Candida albicans</i>	8 ± 1.2	10 ± 1.2	14 ± 0.67	13 ± 1.1	21 ± 0.5
<i>Aspergillus niger</i>	7 ± 1.0	8 ± 1.9	8 ± 0.09	10 ± 1.5	18 ± 0.9

against the gram negative bacteria, that is, *P. aeruginosa* which is common cause of nosocomial infections. The outstanding MIC was observed against *S. aureus* by ethyl acetate (0.31 mg/ml) and *S. typhi* by hexane fraction (0.37 mg/ml). The growth inhibiting capability of the ethyl acetate and aqueous fractions, is an indication for its antibacterial potential and making the *P. integerrima* bark a good candidate for antibacterial drugs. The different fractions of the *Pistacia integerrima* bark do not have significant antifungal activity as shown in Table 3.

The search for new antimicrobial agent is the need of the modern era due the developing resistance of the available antimicrobial agents. Majority of the available antimicrobial agents are expensive and cannot affordable for poor patients therefore the aim of screening crude extract against various microbes specially bacteria and fungi is to find such antimicrobial materials, which are easily available and economically affordable. The larger zone of inhibition exhibited by the methanol, ethyl acetate and aqueous fraction of *P. integerrima* bark as shown in Figure 1 may be due to the presence of active

compounds such as tannins, flavonoids, alkaloids and saponins. The phytochemical study of these fractions revealed the presence of tannins, flavonoids and saponins. The phytochemicals like alkaloids, saponins, flavonoids are the antimicrobial principles of the plant which are responsible for their antimicrobial activities against different pathogens (Haiza, 2000). The results has also supported by Hymete et al. (2005). They reported that flavonoids compounds have antimicrobial activities.

Phytotoxicity assay

The phytotoxic activity of all the fractions is shown in Figure 2. It is clear from the results that all the fractions have herbicidal activities at high concentration however; the outstanding activity was shown by ethyl acetate 90% growth inhibition followed by chloroform 70% growth inhibition and methanol 60% growth inhibition at a concentration of 500 ppm. This reflects that these fractions

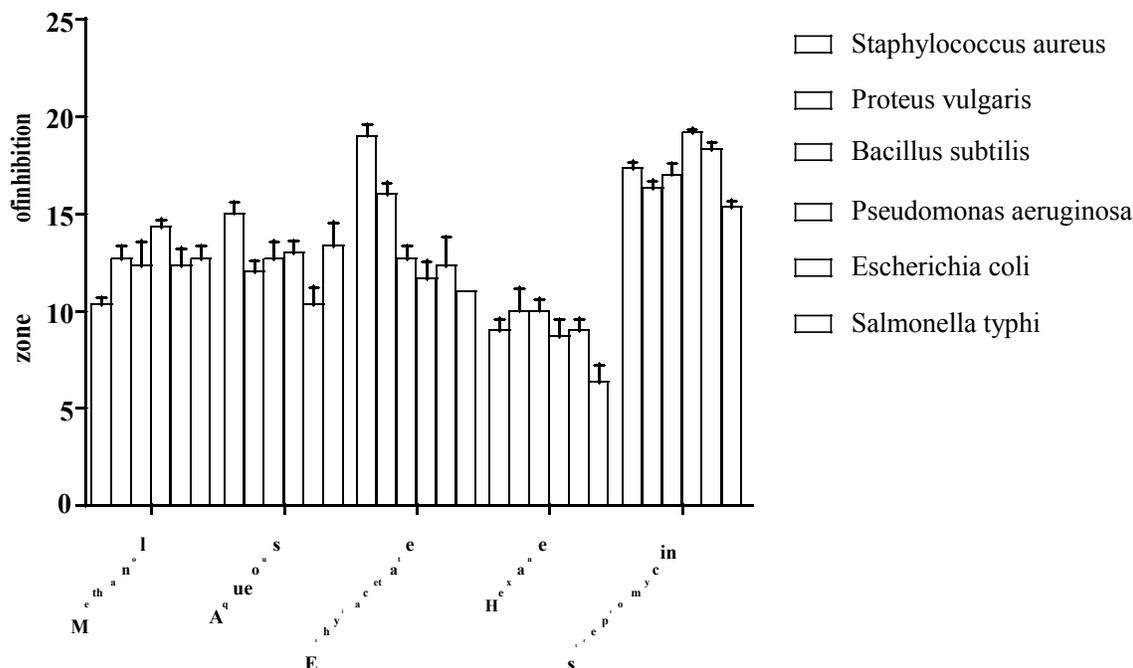


Figure 1. Zone of inhibition of different against selected bacteria.

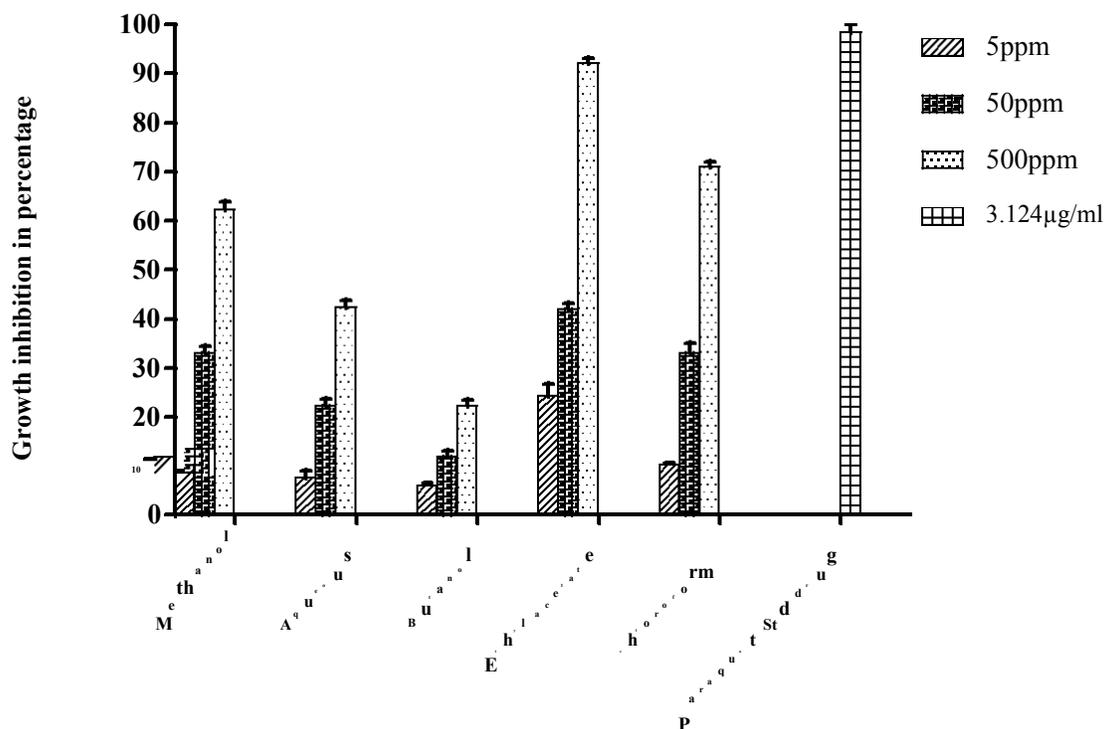


Figure 2. Phytotoxic activity of different fractions against *lemna minor* L.

have active compounds which are responsible for their phytotoxic effect. While comparing all the fractions the ethyl acetate has excellent activities while the hexane fraction has the least. Lemna plants are mono-

cotyledonous plants which are very sensitive to bio active compounds. Lemna assay has been used to detect natural antitumor and phytotoxic compounds (Rehman, 1991). The quality and quantity of agricultural crops is

mostly affected by the presence of extra weeds in the crops. The world economy is strongly affected by the loss of agriculture crops (Piment et al., 2001). The reduction in the growth of these weeds is the prime action for increasing the agriculture crops. Synthetic herbicides are commonly used for the destruction of weeds in agricultural sectors.

However, various factors that restricted the use of synthetic herbicides include water and soil pollution, herbicide-resistant weed populations, and detrimental effects on non-target (Li et al., 2003). In the modern era, more emphasis has been laid on the natural allelochemicals from plants, for weeds control in crops production especially to manage with the problem of weed resistance. It has been proved that the phytotoxicity of plants reduces the growth of weeds without any negative effect on the crops growth and overall yield under normal field condition. It is therefore, assumed on the basis of results that the phytotoxic principle(s) of the bark of the plant could be a significant source of natural herbicides for weeds control.

Conclusion

The result of the study provides a scientific justification to the traditional use of the bark of *P. integerrima* as an antimicrobial agent. Moreover the results showed that the bark has some valuable antimicrobial and phytotoxic compounds which provide a base line for isolation and characterization of innovative compounds. This represents the first preliminary reports on the antimicrobial and phytotoxic activities of *P. integerrima* bark.

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